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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte DAVID BALTIMORE
and
MATTHEW PORTEUS,
Appellants[1]

Appeal 2010-000307
Application 10/656,531
Technology Center 1600

Before ERIC GRIMES, CAROL A. SPIEGEL, and
MELANIE L. McCOLLUM, *Administrative Patent Judges*.

SPIEGEL, *Administrative Patent Judge*.

DECISION ON APPEAL[2]

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[1] The real parties in interest are the California Institute of Technology and the U.S. Government, as represented by the National Institutes of Health, U.S. Department of Health and Human Services (Brief On Appeal Under 37 C.F.R. § 41.37 filed 31 March 2009 ("App. Br.") at 2). This decision also cites to the Examiner's Answer mailed 7 July 2009 ("Ans."), the Reply Brief filed 3 September 2009 ("Reply Br."), and the Specification of the '531 Application ("Spec.").

[2] The two-month period for filing an appeal or commencing a civil action, as recited in 37 C.F.R. § 1.304, or for filing a request for rehearing, as recited in 37 C.F.R. § 41.52, begins to run from the "MAIL DATE" (paper delivery mode) or the "NOTIFICATION DATE" (electronic delivery mode) shown on the PTOL-90A cover letter attached to this decision.

Appellants appeal under 35 U.S.C. § 134(a) from an Examiner's final rejection of claims 21, 28, 99-104, 107, and 108. Claims 43, 109-113, 120-135, and 137-143, the only other pending claims, stand withdrawn from consideration as drawn to a non-elected invention. (App. Br. 2; Ans. 2; Reply Br. 2). We have jurisdiction under 35 U.S.C. § 134. We AFFIRM.

I. Statement of the Case

A. The subject matter on appeal

The subject matter on appeal is directed to vectors and cells containing chimeric nucleases and repair substrates for modifying genes by homologous recombination (gene targeting).

As explained in the '531 Specification, "[g]ene targeting is a process in which the nucleotide sequence at a predetermined genomic site is selectively altered by introduction of an exogenous nucleic acid carrying a desired sequence" (Spec. 17:26-18:1) and "it is generally understood that the selective modification occurs by homologous recombination" (*id.* at 18:1-3). According to the Specification, "creation of a DNA double-stranded break (DSB) in the target gene can increase the frequencies of both direct-repeat recombination and gene targeting several-thousand-fold" (*id.* at 18:13-20 (citations omitted)). DSBs (cleavages) at a site of interest can be achieved by entities which recognize and cleave the site of interest, e.g., nucleases and chimeric nucleases (*id.* at 18:23-29).

The '531 Specification describes using DNA binding domains comprising one or more zinc finger binding domains that each confer recognition of three nucleotides and type II restriction endonucleases, e.g., FokI, cleavage domains to make chimeric nucleases designed to create a DSB at one or more selected sites in the chromosome (*id.* at 21:13-

22:5;25:11-12). Preferably, the chimeric nuclease contains "a nuclear localization signal (NLS) which facilitates the nuclear transport of the chimeric nucleases" (*id.* at 22:28-23:1).

The '531 Specification generally defines a "repair substrate" as "a nucleic acid introduced into a cell for altering a target sequence in chromosomal DNA" (*id.* at 32:4-5) and includes "(i) a polynucleotide sequence that is substantially identical to a region proximal to or flanking a target sequence [homologous sequence]; and (ii) a polynucleotide sequence which replaces the target sequence upon recombination between the repair substrate and the target sequence" (*id.* at 32:12-16). "In a preferred embodiment, the repair substrate is substantially identical, across its entire length except for the sequence changes to be introduced, to a portion of the genome that encompasses both the specific cleavage site and the portions of sequence to be altered" (*id.* at 33:13-16).

According to the '531 Specification, introducing the repair substrate and the chimeric nuclease into a cell in a single vector construct may increase the efficiency for gene targeting (*id.* at 34:3-5).

Claims 21 and 28, the only independent claims on appeal, are illustrative and read (App. Br. 13):

21. A vector comprising:
(1) a nucleic acid encoding a chimeric nuclease that comprises:
(i) a zinc finger DNA binding domain;
(ii) a cleavage domain; and
(iii) a nuclear localization signal; and
(2) a nucleic acid comprising a repair substrate that comprises:

(i) a nucleic acid sequence that is substantially identical to a region flanking a target sequence in chromosomal DNA; and

(ii) a nucleic acid sequence which replaces the target sequence upon recombination between the repair substrate and the target sequence.

28. An isolated mammalian cell comprising:

(a) a chimeric nuclease comprising a zinc finger DNA-binding domain and a cleavage domain; and

(b) a repair substrate comprising

(i) a nucleic acid sequence that is substantially identical to a region flanking a target sequence in endogenous chromosomal DNA; and

(ii) a nucleic acid sequence which replaces the target sequence upon recombination between the repair substrate and the target sequence.

B. The Examiner's rejection

The Examiner has rejected claims 21, 28, 99-104, 107, and 108 under 35 U.S.C. § 103(a) as unpatentable over the combined disclosures of Choulika,[3] Bibikova,[4] and Takeuchi[5] (Ans. 3-8).

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[3] U.S. Patent Application Publication 2002/0107214 A1, *Gene Repair Involving the Induction of Double-Stranded DNA Cleavage at a Chromosomal Target Site*, published 8 August 2002, inventors Andre Choulika and Richard C. Mulligan ("Choulika").

[4] Bibikova et al., *Stimulation of Homologous Recombination through Targeted Cleavage by Chimeric Nucleases*, 21 MOLECULAR AND CELLULAR BIOLOGY 289-297 (January 2001) ("Bibikova").

[5] Takeuchi et al., *Flp recombinase transgenic mice of C57BL/6 strain for conditional gene targeting*, 293 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS 953-957 (2002) ("Takeuchi").

The Examiner found that Choulika teaches chimeric nucleases comprising zinc finger DNA binding domains and cleavage domains from restriction endonucleases, e.g., FokI, and a "targeting DNA", i.e., a repair substrate, comprising a DNA sequence homologous to a region surrounding a specific target sequence of interest in chromosomal DNA and a DNA sequence which repairs the specific target sequence of interest upon recombination between the targeting DNA and the chromosomal DNA (*id.* at 4). The Examiner also found that Choulika discloses a viral vector containing both the chimeric nuclease and the targeting DNA, as well as an isolated mammalian cell containing the vector (*id.*). According to the Examiner, Choulika fails to disclose use of a nuclear localization signal (*id.*).

The Examiner found that Bibikova teaches that chimeric nucleases based on zinc fingers are capable of finding their recognition sites in oocytes, directing specific cleavage, and stimulating local homologous recombination (*id.* at 5). The Examiner found that Takeuchi teaches a vector containing DNA encoding Flp recombinase which is linked to a nuclear localization signal to increase the efficiency of the recombination process (*id.*).

The Examiner concluded that it would have been obvious to modify the viral vector of Choulika by adding a nuclear localization signal as taught by Takeuchi and to transform an isolated mammalian cell with the modified viral vector (*id.* at 6). According to the Examiner, "use [of] a single vector to deliver the nucleic acids encoding the chimeric nucleases and the repair substrate to a host cell [would have been obvious] for the benefit of delivering all the required components in a single vehicle thus reducing the number of transformation/transduction events to one" (*id.* at 7). Further

according to the Examiner, "Choulika et al. already teach a vector that comprises both the nucleic acid encoding the chimeric nuclease and the repair substrate" (*id.*).

C. Appellants' position

Appellants argue that the references teach separating chimeric nuclease-encoding sequences and repair substrates in different vectors to ensure that there is no interference between the two sequences (App. Br. 5-6). Appellants further argue that combining the two sequences into a single vector renders the expression of the sequence encoding the chimeric nuclease and/or availability of the repair substrate unpredictable (App. Br. 7; Reply Br. 3-4). In particular, Appellants argue that Choulika teaches "that targeted DNA and restriction endonuclease-encoding DNA may be carried on separate vectors and the 'and/or' emphasized by the Examiner is a teaching of how the individual vectors can be delivered … Choulika exemplifies only separate vectors" (Reply Br. 6).

Appellants also argue that the cited references fail to establish the predictability of cleaving endogenous target sites (chromosomal targets) with chimeric nucleases as of the instant filing date (App. Br. 7-10; Reply Br. 8-10). Appellants rely on Porteus[6] in support of their position (App. Br. 10; Reply Brief 10-11).

D. Issues

Appellants have argued the appealed claims in two sets -- vector claims 21 and 99-102 and mammalian cell claims 28, 103, 104, 107, and

[6] Matthew H. Porteus & Dana Carroll, *Gene targeting using zinc finger nucleases*, 23 NATURE BIOTECHNOLOGY 967-973 (August 2005) ("Porteus").

108. Therefore, we decide this appeal on the basis of claims 21 and 28. 37 C.F.R. §41.37(c)91)(vii).

At issue is whether the evidence of record supports (i) a conclusion that it would have been obvious to combine a nucleic acid encoding a chimeric nuclease and a nucleic acid repair substrate in the same vector as required by claim 21 and (ii) a reasonable expectation of success that a chimeric nuclease would cleave chromosomal DNA in an isolated mammalian cell; and, if so, whether Appellants have provided evidence that, when weighed with the evidence of obviousness, is sufficient to overcome the *prima facie* conclusion of obviousness.

II. Findings of Fact

The following findings of fact ("FF") are supported by a preponderance of the evidence of record.

A. Choulika

[1] Choulika is directed to methods

> of repairing a specific sequence of interest in chromosomal DNA of a cell comprising (a) inducing in the cell a double stranded break at a site of interest, and (b) introducing into the cell targeting DNA, wherein the targeting DNA comprises (1) DNA homologous to the region surrounding the site of interest and (2) DNA which repairs the specific sequence of interest upon recombination between the targeting DNA and the chromosomal DNA (Choulika ¶ 5 and ¶ 26).

[2] The DSBs are induced by restriction endonucleases or by chimeric nucleases produced by linking DNA binding sequence(s), such as zinc finger binding domains, and DNA cleavage domains, e.g., restriction endonuclease cleavage domains (*id.* at ¶¶ 12-13).

[3] "Targeting DNA *and/or* restriction endonucleases introduced into a cell ... can be inserted into a vector" (*id.* at ¶ 44, emphasis added) according to methods generally known in the art (*id.* at ¶¶ 46-47).

[4] For example,

> A vector comprising a nucleic acid encoding a restriction endonuclease contains all or part of the coding sequence for the restriction endonuclease operably linked to one or more expression control sequences whereby the coding sequence is under the control of transcription signals to permit production or synthesis of the restriction endonuclease. Such expression control sequences include promoter sequences, enhancers, and transcription binding sites. Selection of the promoter will generally depend upon the desired route for expressing the restriction endonuclease. The elements can be isolated from nature, modified from native sequences or manufactured de novo ... and fused together by methods known in the art, ... [*Id. at* ¶ 46.]

[5] Vectors comprising target DNA and/or nucleic acid encoding a restriction endonuclease can be introduced into a cell by conventional means, e.g., transformation (*id.* at ¶ 48).

[6] "Once in the cell, the restriction endonuclease and the vector comprising targeting DNA *and/or* nucleic acid encoding a restriction endonuclease are imported or translocated by the cell from the cytoplasm to the site of action in the nucleus" (*id.* at ¶ 51, emphasis added).

[7] Example 3 describes transforming NIH 3T3 Gap 1 and NIH 3T3 Gap 2 cell lines with different cotransfections of plasmids containing DNA

encoding the restriction enzyme I-SceI and plasmids containing repair/targeting DNA (*id.* at ¶¶ 75-79).

B. Bibikova

[8] According to Bibikova, chimeric nucleases comprising a DNA binding domain made up of three zinc fingers and a nonspecific FokI endonuclease cleavage domain are capable of directing specific cleavage and stimulating local homologous recombination between a target DNA site and an engineered DNA substrate when both the chimeric nuclease and the DNA substrate are injected into frog oocyte nuclei (Bibikova abstract; Fig. 1; 291, ¶ 1; 292, ¶ 3).

[9] Further according to Bibikova, "assembly into chromatin does not prevent access of the nucleases to their targets" (*id.* 295, ¶ 3).

[10] Still further according to Bibikova, "[s]everal additional issues [need] to be addressed to confirm the utility of chimeric nucleases as tools for gene targeting…[i.e.,] demonstrating discrimination against related sequences; proving the efficacy of zinc fingers designed to bind arbitrarily chosen sequences; and testing the cleavage of genuine chromosomal targets" (*id.* at 296, ¶ 7).

C. Takeuchi

[11] Takeuchi teaches adding a nuclear localization signal to the Flp recombinase gene to increase the efficiency of recombination (Takeuchi 954, col. 2, ¶ 2).

D. Porteus (Rebuttal Evidence)

[12] Porteus reviews the state of gene targeting using zinc finger nucleases (chimeric nucleases or "ZFNs") as of 2005 (Porteus title).

[13] Porteus states that "[t]he first genomic locus to be targeted successfully with designed ZFNs was the yellow gene of the fruit fly *D. melanogaster*", citing Bibikova, 161 GENETICS 1169-1175 (2002) (*id.* at 969, first sentence of "ZFNs in model organisms").

[14] According to Porteus, in 2005 "Urnov ... has reported that designed ZFNs can cleave an endogenous human gene in cultured cells and lead to targeted gene replacement in up to 20% of the cells" (*id.* at 970, ¶ 3).

[15] Porteus discusses "[h]oming endonucleases," i.e., "natural genetic elements that catalyze their own duplication into recipient alleles by creating site-specific DSBs that initiate their own genetic transfer by homologous recombination" (*id.* at 969, Box 3).

[16] According to Porteus, one of the interesting features of homing endonucleases is that their expression in mammalian cells does not cause overt cytotoxicity (*id.*).

[17] Porteus states that "optimization of ZFN design must address two key, related issues: specificity and cytotoxicity" (*id.* at 971, last ¶).

[18] According to Porteus, several lines of investigation may offer solutions to ZFN cytotoxicity, including

> tighter control of the level and duration of expression of potentially toxic ZFNs. The loss of targeted cells with time in culture is likely a consequence of continued, although still transient, expression. Because the homologous recombination event is expected to occur rapidly after the target is cut, brief expression of ZFNs should be adequate. [*Id.* at 972, ¶ 2.]

III. Discussion

A. Legal principles

"[T]he test [for obviousness] is what the combined teachings of the references would have suggested to those of ordinary skill in the art." *In re Keller*, 642 F.2d 413, 425 (CCPA 1981). A rejection under 35 U.S.C. § 103(a) is based on the following factual determinations: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art; (3) the differences between the claimed invention and the prior art; and, (4) any objective indicia of non-obviousness. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 399 (2007) (citing *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966)). The "[obviousness] analysis need not seek out precise teachings directed to the specific subject matter of the challenged claim, for a court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ." A person of ordinary skill must be presumed to have some skill, *In re Sovish*, 769 F.2d 738, 743 (Fed. Cir. 1985), and ordinary creativity, *KSR*, 550 U.S. at 418. Furthermore, it is well settled that a reference must be considered in its entirety and that its disclosure is not limited to preferred embodiments or specific working examples contained therein. *In re Fracalossi*, 681 F.2d 792, 794 n.1 (CCPA 1982); *In re Lamberti*, 545 F.2d 747, 750 (CCPA 1976).

All that is required for obviousness under 35 U.S.C. § 103 is a reasonable expectation of success. *In re O'Farrell*, 853 F.2d 894, 904 (Fed. Cir. 1988).

"It is well settled that unexpected results must be established by factual evidence. Mere argument or conclusory statements in the specification does not suffice." *In re De Blauwe*, 736 F.2d 699, 705 (Fed. Cir. 1984).

B. Analysis

Claim 21 recites a vector containing both a nucleic acid encoding a chimeric zinc finger nuclease and a nucleic acid repair substrate. Appellants argue that the references teach separating these two nucleic acids on different vectors to ensure that there is no interference between the two nucleic acid sequences (App. Br. 5-6). However, Appellants have not pointed to, and we do not find, any specific teaching or suggestion in the references indicating that the nucleic acid encoding the chimeric nuclease and the repair substrate nucleic acid should be separated onto separate vectors (see also Ans. 9). To the contrary, Choulika explicitly states that the nucleic acid repair substrate (i.e., targeting DNA) can be on the same vector as the nucleic acid encoding the nuclease (FF 3, 5, 6). Furthermore, to the extent that Example 3 in Choulika exemplifies targeting DNA and nucleic acid encoding the nuclease on different plasmids (vectors) (FF 7), a reference is not limited to its specific working examples. *Fracalossi*, 681 F.2d at 794 n.1; *Lamberti*, 545 F.2d at 750. Moreover, we agree with the Examiner that providing both the chimeric nuclease encoding nucleic acid and the substrate repair nucleic acid on the same vector would be more efficient, i.e., "would reduce the number of transformations/transductions needed to introduce the desired nucleic acids in[to] a host cell" (Ans. 10). Therefore, this argument is not persuasive of patentability.

Appellants also argue that combining the two sequences on the same vector renders the expression of the sequence encoding the nuclease and/or availability of the repair substrate unpredictable (App. Br. 7; Reply Br. 3-4). However, Choulika explicitly teaches that expression of the nucleic acid encoding the nuclease is under the control of transcription signals, such as

promoters sequences and transcription binding sites (FF 4). As noted by the Examiner (Ans. 11), Appellants have not provided any evidence that the repair substrate inhibits expression of the nucleic acid encoding the nuclease. Moreover, to the extent Appellants may be arguing that expression of the sequence encoding the nuclease interferes with the availability of the repair substrate, this is not an issue if the encoding nucleic acid is not being expressed. Vectors, by definition, simply carry nucleic acids to be introduced into host cells; vectors do not themselves express the nucleic acids. Therefore, this argument is not persuasive of patentability of vector claim 21.

To the extent Appellants intend this argument to apply to cell claim 28, we reiterate that Choulika explicitly teaches placing expression of the nucleic acid encoding the nuclease under the control of transcription signals. Indeed, implicit in this teaching of Choulika is that it is within the ordinary skill in the art to control the level and duration of expression of the nucleic acid encoding the chimeric nuclease inside of a cell. To find otherwise is to deny that a person of ordinary skill has some skill, *Sovish*, 769 F.2d at 743, and ordinary creativity, *KSR*, 550 U.S. at 418. Even assuming *arguendo* that the chimeric nuclease contained within the cell of claim 28 was being expressed, Appellants have not provided any evidence establishing that any, or even limited, expression of the nuclease necessarily destroys the cell and/or its usefulness. Therefore, this argument is not persuasive of patentability.

Appellants further argue that the cited references fail to establish the predictability of cleaving endogenous target sites (chromosomal targets) with chimeric nucleases. However, according to Bibikova, "assembly into

chromatin does not prevent access of the nucleases to their [chromosomal] targets" (FF9) and Choulika is expressly directed to methods "of repairing a specific sequence of interest in chromosomal DNA" (FF 1). Therefore, the prior art fairly teaches and/or suggests cleavage of chromosomal targets with chimeric nucleases. Obviousness under 35 U.S.C. § 103 only requires a reasonable expectation of success, not an absolute guarantee. *O'Farrell*, 858 F.2d at 904. In addition, the Examiner pointed out that the '531 Specification states "Chimeric nucleases--fusions between zinc finger binding DNA binding domains and the endonuclease domain of the FokI restriction enzyme ("Fn")--can site-specifically cleave … chromosomal DNA in *Drosophila* (Bibikova, et al., 2002, Genetics, 161:1169-75)"[7] (Ans. 16). Thus, we agree with the Examiner that Choulika's express direction to repair chromosomal DNA (FF 1), Bibikova's express teaching of chromosomal access in a frog system (FF 8-9), and an acknowledged prior art teaching of site-specific chromosomal cleavage in *Drosophila* in the '531 Specification would have provided one of ordinary skill in the art with a reasonable expectation of success in a mammalian system. Appellants' rebuttal evidence does not persuade us otherwise.

According to Appellants, "Porteus clearly teaches that it was not until 2005 that ZFN-mediated targeted integration in mammalian cells was actually shown" and, therefore, "Choulika and Bibikova cannot teach that the claimed methods were predictable, when in 2005, the skilled artisan was stating that the first proof of targeted integration in mammalian cells was available" (Reply Br. 11). However, claim 28 is not a method claim and claim 28 does not positively recite an isolated mammalian cell wherein a

[7] Appellants does not dispute that Bibikova 2002 is prior art (Reply Br. 9).

repair substrate has been integrated into its genome in a targeted (site-specific) location. Similarly, the teachings of Porteus regarding homing endonucleases is not on point (FF 15-16) because the claims on appeal require chimeric zinc finger nucleases. Therefore, on balance, Porteus is insufficient to overcome a reasonable expectation of success that a chimeric nuclease would cleave chromosomal DNA in an isolated mammalian cell provided by the teachings of Choulika and Bibikova to one of ordinary skill in the art.

C. Conclusion

We will sustain the rejection of claims 21, 28, 99-104, 107, and 108 under 35 U.S.C. § 103(a) over Choulika, Bibikova, and Takeuchi. The evidence of record supports a conclusion (i) that it would have been obvious to combine a nucleic acid encoding a chimeric nuclease and a nucleic acid repair substrate in the same vector as required by claim 21 and (ii) a reasonable expectation of success that a chimeric nuclease would cleave chromosomal DNA in an isolated mammalian cell. Appellants' rebuttal evidence, when weighed with the evidence of obviousness, is insufficient overcome the *prima facie* conclusion of obviousness.

IV. Order

Upon consideration of the record, and for the reasons given, it is

ORDERED that the decision of the Examiner to reject claims 21, 28, 99-104, 107, and 108 as unpatentable under 35 U.S.C. § 103(a) over Choulika, Bibikova, and Takeuchi is AFFIRMED; and,

FURTHER ORDERED that no time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(1)(vi).

AFFIRMED

dm

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